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(21) International Application Number: PCT/EP99/03245 (22) International Filing Date: 6 May 1999 (06.05.99) (30) Priority Data: 9801742-9 18 May 1998 (18.05.98) SE (71) Applicant (for all designated States except US): AKZO NOBEL N.V. [NL/NL]; P.O. Box 9300, NL-6800 SB Arnhem (NL). (72) Inventors; and (75) Inventors/Applicants (for US only): AEBI, Robert [CH/CH]; Chemin du Couchant 1, CH-1272 Genolier (CH). LOSA, Riccardo [CH/CH]; En Fagne, CH-1145 Bière (CH). (74) Agent: ANDERSSON, Rolf; Akzo Nobel Surface Chemistry AB, S-444 85 Stenungsund (SE).		(81) Designated States: AU, BR, CA, CN, CZ, JP, KR, NO, NZ, PL, SI, SK, TR, US, ZA, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: USE OF A NATURAL SUBSTANCE CONTAINING THYMOL IN THE MANUFACTURE OF ANIMAL FEED. (57) Abstract <p>The present invention relates to the use of a natural substance containing thymol in the manufacture of an animal feed or a drinkink water supplement for the alleviation, cure or prevention of diseases caused by clostridium sp – particularly clostridium perfringens – in the intestinal tracts of animals, such as poultry and mammals. The natural substance preferably contains a mixture of guaiacol, thymol, eugenol, capsaicin, tannin and at least one cresol. The presence of the mixture also improves the digestibility of the feed and the growth rate of the animals. An animal feed containing 1–500 ppm of the mixture, with the proviso that it does not contain any surface active emulsifying agent, and a drinking water supplement are also described, as well as a premix and a feed additive adapted for use in the manufacture of the animal feed.</p>		

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USE OF A NATURAL SUBSTANCE CONTAINING THYMOL IN THE MANUFACTURE OF ANIMAL FEED

The present invention relates to the use of a natural
5 active substance containing thymol in the manufacture of an
animal feed or a drinking water supplement for the
alleviation, cure or prevention of diseases caused by
clostridium sp - particularly clostridium perfringens - in
the intestinal tracts of animals, such as poultry and
10 mammals. The presence of the substance also improves the
digestibility of the feed and the growth rate of the
animals. The active substance preferably contains a mixture
of guaiacol, thymol, eugenol, capsaicin, tannin and at least
one cresol.

15 Diseases caused by clostridium sp are common in ani-
mal stocks of poultry, pigs, rabbits and rats. There is for
example, a link between the disease necrotic enteritis and
the presence of clostridium perfringens. Necrotic enteritis
is characterized by severe inflammation and sloughing of the
20 intestinal tracts and often occurs together with
coccidiosis.

Many articles have disclosed the amount of clostri-
dium perfringens in the digestive tracts to have consider-
able impact on the health and growth rate of a broiler.
25 Typical symptoms of infected birds are; ruffled feathers,
noticeable depression, loss of appetite, loose/runny dropp-
ings or diarrhoea and a marked reluctance to move. Examples
of such articles are B.S. Bains (1979) "A manual for poultry
diseases" (Ed. Roche, Basel Switzerland); B Köhler, K Vogel
30 and P Starost (1979) "Nekrotisierende und Ulzerative
Enteritis bei Hühner der Mast- und Legerichtung unter Be-
dingungen industriemässiger Geflügelproduktion" (Mh. Vet.-
Med., 32, 704-711); B Köhler, K Vogel, W Witte and H Kühn
(1983) "Vergleich der Ursachen von Hospitalismus durch Cl.
35 perfringens, Staphylococcus aureus und Salmonellen unter den
Bedingungen der industriemässigen Geflügelproduktion und

Möglichkeiten ihrer Bekämpfung", (V. Intern. Tierhyg. Symposium, 25 und 26.05.93, Leipzig, Sammelband der Vorträge, Veterinärmedizinische Fakultät Leipzig); Th. Vissienon, U Johannsen and B Köhler (1994) "Untersuchungen zur Pathologie und Phatogenese der Clostridium-Perfringens-Typ-A-Enterotoxämie des Huhnes. 1. Versuche zur experimentellen Erzeugung der Krankheit, Versuchsansatz, klinisches Bild und Morbiditätsraten", (Mh. Vet.-Med., 49, 23-28); Th. Vissienon, U Johannsen, M Solveig and B Köhler (1994) "Untersuchungen zur Pathologie und Phatogenese der Clostridium-Perfringens-Typ-A-Enterotoxämie des Huhnes. 2. Pathomorphologische und bakteriologische Befunde nach experimenteller intraduodenaler Cl.-perfringens-Typ-A-Infektion" (Sporen und vegetative Keime) und Toxinapplikation (Mh. Vet.-Med., 49, 93-102).

A wide spectrum of antibiotics, such as penicillin, bacitracin and furazolidone are used in treatment. Where the disease is endemic, preventive medication is often added to the animal feed. However, in recent years there has been intense debate about the use of chemical and antibiotic growth promoters and in many countries a ban on this type of feed additives is being considered. Thus, there is an urgent need for agriculture to develop substances which are in line with reliable and generally accepted practice and not of a medicinal nature.

One objective of the present invention is to provide an animal feed or a drinking water supplement which contain natural substances for the cure, prevention or alleviation of the negative effects caused by clostridia on animals in the intestinal tracts. Another objective is to improve the digestibility of the animal feed and thereby hamper or reduce the growth of clostridium sp. A third objective is to reduce the negative effects on the animal growth caused by the presence of clostridium sp.

According to the invention it has been found that these objectives can be achieved by using, in the

manufacture of an animal feed or a drinking water supplement, an effective amount of natural substances - comprised of thymol - for the alleviation, cure or prevention of diseases caused by clostridium sp in the intestinal tracts of animals. By the expression "a natural substance" is in this context understood a substance which consists of compounds occurring in nature and obtained from natural products or through synthesis. The natural substance should preferably contain a mixture consisting of guaiacol, thymol, eugenol, capsaicin, tannin and at least one cresol. Preferably, thymol is the major component of the mixture and most preferably, not less than 50% by weight. Suitably the mixture contains 1-40% by weight of guaiacol, 10-93% by weight of thymol, 1-40% by weight of eugenol, 0.1-20% by weight of capsaicin, 1-75% by weight of tannin and 1-40% by weight of cresol, the amounts being calculated on the total amount of said components. The total amount of these active ingredients may vary within wide limits but is normally from 1 to 500 ppm, preferably between 10 and 100 ppm, calculated on the dry weight of the animal feed. The various active ingredients are all of natural origin and are found in different parts of plants. The animal feed and the drinking water supplement comprising the active ingredients of the invention exhibit excellent effects on necrotic enteritis caused by clostridium perfringens. The presence of the active ingredients also improves the digestibility of the animal feed and reduces the negative effects on the animal growth rate caused by the presence of clostridium sp.

A feed additive containing the active mixture of the present invention for the reduction of coccidiosis has previously been disclosed in WO 96/13175, but this feed additive also contains, as a necessary ingredient, a surface active emulsifying agent. The present invention however, does not require the presence of a surface active emulsifying agent. The invention relates therefore to an animal feed as well as a premix and a feed additive to be used in

the preparation of the animal feed, which animal feed, premix and feed additive contain an effective amount of a mixture consisting of guaiacol, thymol, eugenol, capsaicin, tannin and at least one cresol but lack the presence of a surface active emulsifying agent.

In a preferred embodiment of the invention the animal feed may contain 1-5 ppm of cresol, 1-5 ppm guaiacol, 5-50 ppm of thymol, 1-5 ppm of eugenol, 0.1-2 ppm capsaicin and 1-20 ppm tannin. In addition hereto, the animal feed can also contain other chemical compounds of plant origin such as flavourings in an amount of 0.5-50 ppm, calculated on the dry weight of the animal feed. Examples of suitable flavourings are 0.05-0.5 ppm creosol, 0.1-5 mg anethole, 0.1-2 ppm of deca-, undeca- and/or dodecalactones, 0.1-2 ppm quino-
leine, 0.1-2 ppm ionones and/or irone, 0.05-1 ppm gingerol, 0.05-1 ppm piperidine, 0.05-1 ppm propylidene and/or butylidene phthalides and 0.1-5 ppm amyl and/or benzyl salicilate. The incorporation of active ingredients into the animal feed is usually carried out by preparing a premix of the active ingredients and other suitable additives. Such a premix may comprise 2-10% by weight of the active mixture, 0-40% by weight of other conventional additives, such as flavourings, and 50-98% by weight of any conventional absorbing support. The support may contain, for example, 40-50% by weight of wood fibres, 8-10% by weight of stearin, 4-5% by weight of curcuma powder, 4-5% by weight of rosemary powder, 22-28% by weight of limestone, 1-3% by weight of a gum, such as gum arabic, 5-50% by weight of sugar and/or starch and 5-15% by weight of water.

This premix is then mixed with vitamins, enzymes, mineral salts, ground cereals, protein-containing components, carbohydrate-containing components, wheat middlings and/or brans in the preparation of an animal feed additive which contains 0.2-5% by weight of the premix. The animal feed additive is then finally added to the feed in such quantities that the feed will contain 1-500 ppm, preferably 10-100 ppm, of the active mixture. The animal feed additive

normally constitutes 0.3-3.5% by weight of the animal feed.

The animal feed according to the invention usually contains, calculated on the dry weight of the feed, the following ingredients:

- 5 a) 0-80%, preferably 10-70%, by weight of cereals,
 - b) 0-30%, preferably 1-12%, by weight of feed fat,
 - c) 0-85%, preferably 10-50%, by weight of protein
containing nutritious substances of a type other than
cereals, and
 - 10 d) 1-500 ppm, preferably 10-100 ppm, of the mixture.
- The total amounts of a)-d) are preferably at least 80% by weight.

When preparing the animal feed, the animal feed additive can be mixed with the dry ingredients consisting of
15 cereals, such as ground or crushed wheat, oats, barley, maize and rice; vegetable protein feed based on e.g. rape-seed, soya bean and sunflower; animal protein feed, such as blood meal, meat and bone meal and fish meal; molasses; and milk products, such as various milk powders and whey
20 powders. After mixing all the dry additives, the liquid ingredients and ingredients, which after heating become liquid, can be added. The liquid ingredients may consist of lipids, such as fat, for example slaughter fat and vegetable fat, optionally liquefied by heating, and/or of carboxylic
25 acids, such as a fatty acid. After thorough mixing, a mealy or particulate consistency is obtained, depending on the degree of grinding of the ingredients. To prevent separation during storage, water should preferably be added to the animal feed, which then is subjected to a conventional
30 pelletising, expanding or extruding process. Any excess water can be removed by drying. If desired, the resulting granular animal feed can also be crushed to a smaller particle size.

Within the scope of the invention, it is also possible to produce a suspension of the animal feed. This is
35 especially convenient if the feed is prepared for immediate consumption.

The drinking water supplement may contain 2-90% by weight, preferably 10-50% by weight, of the natural substance. Preferably the natural substance contains a mixture of guaiacol, thymol, eugenol, capsaicin, tannin and at least one cresol and preferably thymol is the major component of the mixture and most preferably not less than 50% by weight. Suitably the mixture contains 1-40% by weight of guaiacol, 10-93% by weight of thymol, 1-40% by weight of eugenol, 0.1-20% by weight of capsaicin, 1-75% by weight of tannin and 1-40% by weight of cresol, the amounts being calculated on the total amount of said components. Beside the natural substance the supplement may also contain 10-98% by weight of a large number of other ingredients. Common ingredients are mineral salts, vitamins, flavourings, water-soluble or water-dispersable carriers, such as sugars, powdered milk, milk-by-products and cellulose derivatives, and stabilisers, such as water-soluble or water-dispersable polymers. When preparing the drinking water, the supplement is normally added to the water in such an amount that the concentration of the natural substance becomes 1-500 ppm.

The present invention will now be further illustrated by the following Examples.

Example 1

The efficacy of the mixture of the invention to reduce the occurrence of clostridia and in particular *Clostridium perfringens* and to increase the animal growth rate was measured. The tests were performed on young fowls, which were put on commercial diets with the following basic compositions.

Table 1.

Feeding program	Starter feed	Grower feed	Finisher feed
Crude protein, %	23.0	21.5	21.0
Crude fat, %	8.9	10.5	10.5
Crude fibre, %	3.0	3.1	3.4
Crude ash, %	5.5	5.0	4.8
MJME, kg	13.0	13.4	13.4

These diets were formulated by mixing suitable amounts of wheat, soyabean meal, peas, rapeseed meal, bone meal and oil. To the diets administered to a control group were also added zinc-bacitracin, a traditional growth promoter in the ratio of 20 mg per kg of the feed, and to the diets administered to the experimental group 50 mg per kg of the feed of a mixture consisting of 3 mg cresols, 3 mg guaiacol, 30 mg thymol, 3.5 mg eugenol, 0.5 mg capsaicin and 10 mg tannin, the main component in this mixture being thymol. To the feed of both groups was also added 5 mg ammonium-maduramicin per kg of feed. The fowls were divided into two groups and fed for 39 days on the two feed compositions. The following results were obtained.

Table 2.

	Experimental group	Control group
Animals, no	21 600	32 400
Chicken, weight at start, g	35.5	35.9
Weight, 39 days, g	2 056	2 048
Growth/day, g	51.79	51.59
Feed conversion rate	1.71	1.73

The results clearly demonstrate the growth rate of the fowls to be improved by the addition of the mixture according to the invention in comparison to the growth obtained with the addition of the traditional growth promoter,

zink-bacitracin, also known to limit the development of clostridia. Furthermore, the feed conversion rate (feed intake/increase in weight) was also improved when the animal feed according to the invention was used.

5 The occurrence of clostridia in the digestive tract was also determined. Samples of ileum, caecum and colon were taken from both groups on days 5, 18 and 32 of the trial. Each trial examination group consisted of 10 fowls chosen at random. The presence of Clostridia and of Clostridium per-
10 fringens were isolated and confirmed by testing for the production of toxins. The following results were obtained.

Table 3. Percentage of positive sample of clostridia and Clostridium perfringens

	Experimental group	Control group
Whole digestive tract		
Total clostridia	79	92
Clostridium perfringens	26	52
Ileum		
Clostridium perfringens	33	50
Caecum		
Clostridium perfringens	23	50
Colon		
Clostridium perfringens	20	57

15 **Table 4. Percentage of positive samples of Clostridium perfringens on different days**

Day	Experimental group	Control group
5	33	46
18	20	30
32	23	80

The occurrence of clostridia in general and of *Clostridium perfringens* in particular is essentially lower when the mixture of the invention is added to feed when compared to an addition of zinc-bacitracin.

5 **Example 2.**

The antimicrobial activity of the mixture of the invention towards *Clostridium perfringens* was determined in the following manner.

10 *Clostridium perfringens* ATCC 13124 (10.6 cfu/ml) was added to cultures containing different amounts of the mixture disclosed in Example 1 and the cultures were left to incubate. After incubation a first reading was taken before agitation. The growth of the cultures of the invention was also compared to the growth of the controls by visual comparison. Where the culture remained clear this indicated no growth. In case of doubtful samples (usually those containing high concentrations of the mixture) a subculture was prepared from the broth into gelose to confirm, if any growth had taken place. The following results were obtained.

	Concentration of the mixture according to the invention, ppm						
	500	250	125	100	75	50	0
Growth	-/-/-	-/-/-	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+
Control							
Comparison, Invention/Control	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	-/-/-	

20

The symbol "-" indicates no growth or, in the comparison, lower growth than the control, while "+" indicates growth or, in the comparison, more growth than the control.

25 From the results it is evident that the mixture of the invention has an in vitro bacteriostatic effect on *Clostridium perfringens*.

C L A I M S

1. Use of a natural substance containing thymol in the
5 manufacture of an animal feed or a drinking water supplement
 for the alleviation, cure or prevention of diseases caused
 by clostridium sp in the intestinal tracts of animals.
2. Use according to claim 1, wherein the natural sub-
 stance contains a mixture of guaiacol, thymol, eugenol,
10 capsaicin, tannin and at least one cresol.
3. Use according to claims 1 or 2, wherein the mixture
 contains 1-40% by weight of guaiacol, 10-93% by weight of
 thymol, 1-40% by weight of eugenol, 0.1-20% by weight of
 capsaicin, 1-75% by weight of tannin and 1-40% by weight of
15 cresol, the amounts being calculated on the total amount of
 said components.
4. Use according to claim 3, wherein the animal feed
 contains
 - a) 10-70% by weight of cereals,
 - 20 b) 1-12% by weight of feed fat,
 - c) 10-50% by weight of protein containing nutritious
 substances of a type other than cereals, and
 - d) 1-500 ppm of the mixture.
5. Use according to any one of the claims 1-4 for the
25 alleviation, cure or prevention of necrotic enteritis.
6. Use according to any one of the claims 1-5, wherein
 the animal feed contains flavourings in an amount of 0.5-50
 ppm.
7. Use according to any one of the claims 1-6 in the
30 manufacture of a poultry feed.
8. Animal feed, characterized in, that it contains 1-500
 ppm, preferably 10-100 ppm, calculated on the dry weight of
 the animal feed, of a mixture consisting of guaiacol,
 thymol, eugenol, capsaicin, tannin and at least one cresol,
35 with the proviso that it does not contain any surface active
 emulsifying agent.
9. Animal feed according to claim 8, characterized in,

that the mixture consists of 1-40% by weight of guaiacol, 10-93% by weight of thymol, 1-40% by weight of eugenol, 0.1-20% by weight of capsaicin, 1-75% by weight of tannin and 1-40% by weight of cresol, the amounts being calculated on the total amount of said components.

10. Animal feed according to claims 8 or 9, characterized in, that it contains

- a) 10-70% by weight of cereals,
- b) 1-12% by weight of feed fat, and
- 10 c) 10-50% by weight of protein containing nutritious substances of a type other than cereals.

11. Animal feed according to any one of claims 8-9, characterized in, that it contains flavourings in amounts of 0.5-50 ppm, calculated on the dry weight of the animal feed.

15 12. A premix, characterized in, that it contains

- i) 2-10% by weight of a mixture of guaiacol, thymol, eugenol, capsaicin, tannin and at least one cresol,
- ii) 0-30% by weight of other conventional additives, including flavourings, and
- 20 iii) 50-98% by weight of an absorbing support, with the proviso that it does not contain any surface active emulsifying agents.

13. A premix according to claim 12, characterized in, that the mixture contains 1-40% by weight of guaiacol, 10-93% by weight of thymol, 1-40% by weight of eugenol, 0.1-20% by weight of capsaicin, 1-75% by weight of tannin and 1-40% by weight of cresol, the amounts being calculated on the total amount of said components.

14. An animal feed additive, characterized in, that it contains 0.2-5% by weight of the premix in claims 12 or 13, 80-99.8% by weight of vitamins, enzymes, mineral salts, ground cereals, protein components, carbohydrate components, wheat middlings and/or bran, with the proviso that it does not contain any surface active emulsifying agent.

15. Animal feed additive according to claim 14, characterized in, that the mixture consists of 1-40% by weight of guaiacol, 10-93% by weight of thymol, 1-40% by weight of

eugenol, 0.1-20% by weight of capsaicin, 1-75% by weight of tannin and 1-40% by weight of cresol, the amounts being calculated on the total amount of said components.

16. Use of the animal feed additive or the premix according to claims 12 or 15 in the preparation of an animal feed additive in accordance with claims 6-9.

17. A drinking water supplement, characterized in, that it contains 2-90% by weight of the natural substance as defined in claims 1, 2 or 3 and 10-98% by weight of
10 vitamines, mineral salts, flavourings, water soluble carriers, such as sugar, milk powder, milk-by-products and cellulose derivatives, and stabilisers, such as water-soluble or water-dispersable polymers.

INTERNATIONAL SEARCH REPORT

International Application No

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A23K1/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 13175 A (CRINA S.A.) 9 May 1996 (1996-05-09) cited in the application the whole document	1-17
X	US 5 558 889 A (ROSSI) 24 September 1996 (1996-09-24) column 2, line 7 - line 50; examples -/-	8,10-15

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>M.D. PIERSON ET AL.: "Inhibition of Clostridium Botulinum by Antioxidants and Related Phenolic Compounds in Comminuted Pork"</p> <p>JOURNAL OF FOOD SCIENCE., vol. 47, 1982, page 1926-1929, 1935 XP002113955</p> <p>INSTITUTE OF FOOD TECHNOLOGISTS. CHICAGO., US ISSN: 0022-1147 page 1928</p>	1
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Information on patent family members

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